American Journal of Hematology 61:265–267 (1999)

Prevalence of Factor V Leiden, Prothrombin G20210A, and MTHFR C677T Mutations in a Greek Population of Blood Donors

T. Antoniadi,[1] T. Hatzis,[2] C. Kroupis,[1] E. Economou-Petersen,[3] and M.B. Petersen[1]*

[1]Department of Genetics and Molecular Biology, Mitera Maternity and Surgical Center, Marousi, Greece
[2]Department of Hematology, Mitera Maternity and Surgical Center, Marousi, Greece
[3]Drakopoulio Blood Bank Center, Athens, Greece

The pathogenesis of venous thrombosis involves the interaction of genetic and environmental factors. In order to estimate the frequency of the factor V Leiden, the prothrombin G20210A, and the MTHFR C677T mutations in the Greek population, we analyzed 160 healthy Greek blood donors by PCR amplification and detected allele frequencies of 2.5%, 2.2%, and 35.3%, respectively. The allele frequencies were compared with reported frequencies of other populations of southern Europe. The identification of these common genetic risk factors for thrombosis should enable easy DNA diagnosis and carrier detection in a high proportion of cases and will contribute to a better understanding of the interaction of genetic and environmental risk factors. Am. J. Hematol. 61:265–267, 1999. © 1999 Wiley-Liss, Inc.

Key words: thrombophilia; factor V; prothrombin; methylenetetrahydrofolate reductase

INTRODUCTION

The pathogenesis of venous thrombosis is complex, involving the interaction of genetic and environmental factors. A well-established genetic predisposition to venous thrombosis is a single point mutation in the gene encoding coagulation factor V (G1691A or factor V Leiden) [1], with a 5–10-fold increased risk of thrombosis in heterozygotes and a 50–100-fold increased risk in homozygotes. Previous studies have reported a factor V Leiden carrier frequency in Europeans of 8.8%, whereas outside Europe the mutation is very rare [2]. More recently, a second common genetic risk factor has been identified, the G20210A variation in the 3′-untranslated region of the prothrombin gene associated with elevated plasma prothrombin levels and an almost threefold increased risk of venous thrombosis [3]. A carrier frequency around 3.0% has been detected in southern Europe, nearly twice as high as in northern Europe, whereas the prothrombin variant is very rare in non-Caucasians [4].

Hyperhomocysteinemia has also been shown to be a risk factor for venous thrombosis [5]. A common mutation (C677T) in the methylenetetrahydrofolate reductase (MTHFR) gene, which renders the MTHFR enzyme thermolabile, is in the homozygous state associated with mild hyperhomocysteinemia [6]. The C677T MTHFR mutation was therefore suggested as a candidate genetic risk factor for venous thrombosis [6], which was actually found in some studies [7], while other studies failed to show an association [8]. These inconsistencies could be explained by differences in genetic background or environmental risk factors between the study populations. An allele frequency of C677T has been found between 2.0% and 54.5% in different populations of the world [9]. The purpose of the present study was to estimate the frequency of the factor V Leiden, the prothrombin G20210A, and the MTHFR C677T mutations in the Greek population.

MATERIALS AND METHODS

Blood samples were collected from 160 voluntary, healthy Greek blood donors visiting the Drakopoulio

*Correspondence to: Dr. M.B. Petersen, Department of Genetics and Molecular Biology, Mitera Maternity and Surgical Center S.A., Kifissias Avenue and 6 Erythrou Stavrou Street, GR-151 23 Marousi, Greece.

Received for publication 12 February 1999; Accepted 7 April 1999

TABLE I. Prevalence of Factor V Leiden, Prothrombin G20210A, and MTHFR C677T Mutations in 160 Greek Blood Donors

	Factor V G1691A	Prothrombin G20210A	MTHFR C677T
Heterozygotes	8	7	87
Homozygotes Mutant	0	0	13
Homozygotes Normal	152	153	60
Total	*160*	*160*	*160*
Carrier Frequency	5.0%	4.4%	62.5%
Allele Frequency	2.5%	2.2%	35.3%
95% CI	*0.8–4.2%*	*0.6–3.8%*	*30.1–40.5%*

Blood Bank Center in Athens. All the blood donors had to answer and sign a standard questionnaire produced by the National Blood Banking Committee of the Greek Ministry of Health. The blood donors originated from different parts of the country. The mean age was 29.1 years, range 20–60 years. The 160 individuals were 116 males (mean age 28.7 years) and 44 females (mean age 30.2 years).

Molecular diagnosis was performed after polymerase chain reaction (PCR) amplification of genomic DNA, restriction enzyme digestion, and agarose gel electrophoresis, using primers and restriction enzymes as described for the factor V Leiden [10], prothrombin G20210A [11], and MTHFR C677T [6] mutations.

RESULTS

The mutation frequencies in the 160 Greek blood donors are shown in Table I. The factor V and prothrombin mutations were detected in 8 and 7 blood donors, giving allele frequencies of 2.5% (95% confidence interval 0.8–4.2%) and 2.2% (95% CI 0.6–3.8%), respectively. The allele frequency of the MTHFR mutation was 35.3% (95% CI 30.1–40.5%). The distribution of genotypes were as predicted by the Hardy-Weinberg equation except for the MTHFR C677T ($0.010 < P < 0.025$).

DISCUSSION

The allele frequency of factor V Leiden (2.5%) in the Greek blood donors is consistent with the previously reported 4.2% in a sample of 203 individuals originating from all over the country but of unspecified ascertainment [12]. An allele frequency of 7.0% was reported in Greek Cypriots (187 referrals for diagnosis of hemoglobinopathies) [2], and part of the difference might be due to the different ascertainment of the studied populations, probably excluding some people with previous thrombosis from being blood donors. It should also be kept in mind that the Greek Cypriot population of Cyprus is not identical to the Hellenic population of mainland Greece. Allele frequencies of the factor V Leiden mutation of 1.3% and 1.7% have been reported in Italian [13] and Spanish [10] populations, respectively. Haplotype analyses of Caucasian individuals homozygous for the Leiden mutation support a single mutational event and provide a powerful tool to investigate its evolutionary history [14].

The prothrombin G20210A and MTHFR C677T mutations have not previously been reported in Greeks. The geographic distribution of the prothrombin G20210A variant showed a nearly twice as high prevalence in southern Europe as compared to northern Europe, while it appeared very rare among Africans and Asians [4]. It seems likely that the geographical distribution is due to a founder effect as has been shown for the factor V Leiden. The MTHFR C677T mutation has a relatively high frequency throughout the world, and the geographical pattern of the allele frequency supports the hypothesis that it is a risk factor for vascular disease and neural tube defects. One possible explanation for this high frequency could be a heterozygote or homozygote mutant advantage of so far unknown kind. We found an allele frequency of 35.3% in Greeks compared to 44.8% and 54.5% in Italian and Spanish people, respectively [9]. As reported for the prothrombin G20210A variant the MTHFR C677T mutation seems to have an increasing north-to-south cline, but the amount of data available is still small [9].

The point mutations discussed here represent common genetic risk factors for vascular diseases including obstetrical complications that are related to inadequate maternal-fetal circulation such as severe preeclampsia, abruptio placentae, fetal growth retardation, and stillbirth [15]. Population-based studies on the prevalence of these mutations will contribute to a better understanding of the interaction between genetic and environmental risk factors underlying the mentioned disorders.

REFERENCES

1. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature (London) 1994;369:64.
2. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995;346:1133.
3. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698.
4. Rosendaal FR, Doggen CJM, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998;79:706.
5. den Heijer M, Koster T, Blom HJ, Bos GMJ, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 1996;334:759.
6. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen

R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10: 111.
7. Arruda VR, von Zuben PM, Chiaparini LC, Annichino-Bizzacchi JM, Costa FF. The mutation Ala677Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. Thromb Haemost 1997;77:818.
8. Brown K, Luddington R, Baglin T. Effect of the MTHFRC677T variant on risk of venous thromboembolism: interaction with factor V Leiden and prothrombin (F2G20210A) mutations. Br J Haematol 1998;103:42.
9. Pepe G, Camacho Vanegas O, Giusti B, Brunelli T, Marcucci R, Attanasio M, Rickards O, De Stefano GF, Prisco D, Gensini GF, Abbate R. Heterogeneity in world distribution of the thermolabile C677T mutation in 5,10-methylenetetrahydrofolate reductase. Am J Hum Genet 1998;63:917.
10. Garcia-Gala JM, Alvarez V, Pinto CR, Soto I, Urgelles MF, Menendez MJ, Carracedo C, Lopez-Larrea C, Coto E. Factor V Leiden (R506Q) and risk of venous thromboembolism: a case-control study based on the Spanish population. Clin Genet 1997;52:206.
11. Ripoll L, Paulin D, Thomas S, Drouet LO. Multiplex PCR-mediated site-directed mutagenesis for one-step determination of factor V Leiden and G20210A transition of the prothrombin gene. Thromb Haemost 1997;78:960.
12. Chaida C, Gialeraki A, Tsoukala C, Mandalaki T. Prevalence of the FVQ506 mutation in the Hellenic population. Thromb Haemost 1996; 76:127.
13. Mannucci PM, Duca F, Peyvandi F, Tagliabue L, Merati G, Martinelli I, Cattaneo M. Frequency of factor V Arg506Gln in Italiens. Thromb Haemost 1996;75:694.
14. Zivelin A, Griffin JH, Xu X, Pabinger I, Samama M, Conard J, Brenner B, Eldor A, Seligsohn U. A single genetic origin for a common Caucasian risk factor for venous thrombosis. Blood 1997;89:397.
15. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing JB. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999;340:9.